*National University of Ireland, Galway*

**Annual Student Report to GRC**

*To be completed by all research (PhD, MD and Research Masters) students every year and submitted to the GRC in advance of the annual review meeting with the GRC.*

|  |  |
| --- | --- |
| **Name of Student** | Sofiia Tretiak |
| **Student ID** | 20249997 |
| **Year of Study** | 2 |
| **PhD / MD / Research Masters** | PhD |
| **Discipline / School** | Botany and Plant Sciences |
| **Full or Part Time** | Full time |
| **Name of Supervisor(s)** | Zoe Popper |
| **Period covered by report** | June 2021- June 2022 |

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Description of work completed during this period:**  Additional instructions may be supplied by your supervisor, GRC, Discipline or School as to the level of detail required. It is expected that you would address the following:  Background  Research Question / Objectives / Hypothesis  Methodology  Results / Findings  Discussion   1. **Background**   **1.1 Naturally-derived biologically active compounds and their applications**  Biologically active compounds derived from the natural sources has an increasing interest in the cosmetic, medical, pharmaceutical, food and horticulture applications (Cikoš et al., 2018; Hentati et al., 2020; Pitkänen et al., 2018; Stengel et al., 2011b). Marine macroalgae represent one of the most sustainable non-animal-derived sources of such compounds including polysaccharides, proteins, lipids, polyphenols, and others (Hentati et al., 2020; Stengel et al., 2011b; Stiger-Pouvreau et al., 2016). Main applications of polysaccharides are largely dependent on their physical properties as they are hydrophilic, water-soluble, and have gelling properties (Stengel et al., 2011a).  Brown seaweeds are the second largest group of macroalgae after red seaweeds (Bhakti & Avinash, 2019) and contain different types of polysaccharides such as alginates, laminarans and fucoidans (Abraham et al., 2019; Bhakti & Avinash, 2019). Alginates are commonly used as a food ingredients, whereas laminarans and fucoidans are promising bioactive compounds with pharmaceutical, cosmetics and food applications (Ahmed et al., 2014; Rioux et al., 2007).  Pure forms of bioactive substances are highly valuable for commercial applications, thus, setting optimal separation and purification conditions are of a great industrial importance (Akin et al., 2012; Le Bourvellec et al., 2005). However, during the polysaccharides extraction polyphenols are commonly co-extracted and contribute to the astringency and bitter taste that limits their use e.g. in some food applications (Akin et al., 2012; Le Bourvellec et al., 2005). Established methods for separating polysaccharides from polyphenols are often costly, affect the bioactive compound production yield or are not food-friendly, thus, development of environmentally sustainable extraction and purification techniques, using nontoxic solvents for further use in the food applications are of a high interest (Acosta et al., 2014; Akin et al., 2012; Cikoš et al., 2018; Giacobbo et al., 2017; Hentati et al., 2020; Kadam et al., 2013a).  **1.2 Extraction of polysaccharides: obstacles and opportunities**  Pure forms of bioactive substances are highly valuable for commercial applications, thus, setting optimal separation and purification conditions are of a great industrial importance (Akin et al., 2012; Le Bourvellec et al., 2005). However, during the polysaccharides extraction polyphenols are commonly co-extracted and contribute to the astringency and bitter taste that limits their use e.g. in some food applications (Akin et al., 2012; Le Bourvellec et al., 2005). Established methods for separating polysaccharides from polyphenols are often costly, affect the bioactive compound production yield or are not food-friendly, thus, development of environmentally sustainable extraction and purification techniques, using nontoxic solvents for further use in the food applications are of a high interest (Acosta et al., 2014; Akin et al., 2012; Cikoš et al., 2018; Giacobbo et al., 2017; Hentati et al., 2020; Kadam et al., 2013a).  To improve polysaccharides extraction from seaweeds additional steps prior to the main extraction process are commonly applied. These steps are conducted to minimize co-extraction of compounds with similar solubility and to increase the cell wall disruption for easier polysaccharides transfer to the extraction solvent (Dobrinčić et al., 2020). However, commonly used solvents such as methanol, chloroform, and acetone among others are toxic (Dobrinčić et al., 2020; Hentati et al., 2018), thus, the development of sustainable and efficient extraction technology is of high relevance (Dobrinčić et al., 2020; Xu et al., 2017).  Some of the novel extraction techniques that have shown an effect on cell wall integrity and polysaccharide yield include microwave-assisted extraction (MAE)(M. Garcia-Vaquero et al., 2017; Marco Garcia-Vaquero et al., 2020; Hentati et al., 2020; Yuan & Macquarrie, 2015), ultrasound-assisted extraction (UAE)(Garcia-Vaquero et al., 2020; Hentati et al., 2020), enzyme-assisted extraction (EAE)(Hentati et al., 2020), pressurized-liquid extraction (PLE)(Becerra et al., 2015; Hentati et al., 2020; Otero et al., 2018), and autoclave-based hydrothermal-assisted extraction (HAE) (Allahgholi et al., 2020; Garcia-Vaquero et al., 2019; Rajauria et al., 2010). Following techniques were employed and investigated against the conventional methods of polysaccharides extraction.   * 1. **Extraction of polysaccharides: choosing the right species**   Therefore, with the idea of the MINERVA project about maximising the seaweed biomass utilisation, two seaweed species: *Ascophyllum nodosum* and *Saccharina latissima* (previously known as) were chosen. Thus, during the first cycle of extraction fucoidans and laminarans can be isolated with further applications in cosmetics (by Matis Ltd, Iceland – MINERVA project partner), in horticulture (by Sofiia Tretiak at NUIG) – for the immunostimulatory spray tests on the strawberry plants against the *Rhizopus* fungi. The residue can then further be used for the alginate extraction (by Sofiia Tretiak at NUIG) – for an advisory methodology development for the industrial scale polysaccharide extraction or for a food fiber production (cellulose+alginate) (Cebercolloids Ltd. Ireland– MINERVA project partner).   * 1. **Extraction of polysaccharides: choosing the right solvent and species**      1. **Alginate**   Alginate is a main component of the brown seaweed cell walls as well as it also appears in the intercellular matrix. Alginate is abundant in most brown macroalgae, however its content is species and season specific (Kravchenko et al., 2018; Rhein-Knudsen et al., 2015). Thus, in the *Ascophyllum* spp. - one of the main commercially utilized seaweeds for alginate production (Fenoradosoa et al., 2010; Rhein-Knudsen et al., 2015), alginate content is estimated at around 18-24 % to a seaweed dry weight (Montes et al., 2021; Yuan & Macquarrie, 2015b).  There are various methods of the alginates extraction, however, most of them rely on extracting insoluble alginate through its soluble counterpart – sodium alginate (as described by Calumpong et al., 1999). In this method insoluble calcium-alginates, that contains in brown seaweeds, is converted into soluble sodium alginates that are further extracted as either alginic acid or calcium alginate (Calumpong et al., 1999; Fenoradosoa et al., 2010).   * + 1. **Fucoidans and laminarans**   Laminarin is a food reserve of brown seaweeds and is located in vacuoles in algal cells (Kadam, et al., 2015). Laminarin is absent during the period of fast growth in spring, but in autumn and winter, it varies between 4-6% of the seaweed dry weight in *Ascophyllum nodosum* (Kadam, et al., 2015; Kadam, et al., 2015). Fucoidan is a sulphated polysaccharide also abundant in brown seaweeds (Fletcher et al., 2017b). Its content and structure is species-specific (Fletcher et al., 2017b) and in *Ascophyllum* its content can rich up to 16% of the seaweed dry weight (Yuan & Macquarrie, 2015a). Overall, these two groups of polysaccharides can be extracted with acid or water (Kadam et al., 2013b).  Sulfated polysaccharides and laminarans are promising bioactive compounds with pharmaceutical: as a component in the drug delivery systems (Cunha & Grenha, 2016), antitumour (Kuda et al., 2005), antioxidant(Choi et al., 2007), cosmetics and food applications (Ahmed et al., 2014; Rioux et al., 2007).  **1.5 Separation of the polysaccharides from the co-extracted phenolics**  In the process of extracting polysaccharides from brown seaweeds polyphenols are commonly co-extracted and contribute to the astringency and bitter taste that limits their use e.g. in some food applications (Akin et al., 2012; Le Bourvellec et al., 2005). Phlorotannins are the most abundant polyphenolics in brown seaweeds among many others (Gómez-Guzmán, 2018; Hentati et al., 2020). Established methods for separating polysaccharides from polyphenols are often costly, affect the bioactive compound production yield or are not food-friendly, thus, development of environmentally sustainable extraction and purification techniques, using nontoxic solvents for further use in the food applications are of a high interest (Acosta et al., 2014; Akin et al., 2012; Cikoš et al., 2018; Giacobbo et al., 2017; Hentati et al., 2020; Kadam et al., 2013a).  **1.5.1 Polyvinylpolypyrrolidone (PVPP)**  Insoluble polyvinylpolypyrrolidone (PVPP) has been known to bind phenolic compounds and due to this activity it is widely used in beer, wine and juice industries to clarify beverages (Doner et al., 1993; Magalhães et al., 2010; McMurrough et al., 1995; Siebert & Lynn, 1998). A study of (Toth & Pavia, 2001) was the first time when insoluble polyvinylpolypyrrolidone (PVPP) was effectively used to specifically remove tannins and from *Ascophyllum* *nodosum* extract. Therefore, in our study we used PVPP in different dosage with the aim to separate phenolic compounds from the polysaccharides in the *Ascophyllum* *nodosum* extract.  **1.5.2 Isinglass**  Another agent, that is traditionally used in brewing industry for several hundred years is isinglass (Hickman et al., 2000). It is obtained from the dried swim bladders of tropical marine fish and primarily consists of the collagen protein (Hickman et al., 2000; Leather et al., 1994). The traditional use of isinglass in beer clarification was relying on charge interractions between collagen and yeast or polyphenols. Thus, electrostatically attracted yeast and polyphenols formed complexes with collagen and further precipitated as the sediment (Hickman et al., 2000). However, isinglass collagen is thermolabile as it denatures at 29°C and consequently is not effective in clarifying solutions at temperatures above its denaturation point (Hickman et al., 2000).  **1.5.3 Bovine Serum Albumin (BSA)**  Bovine serum albumin (BSA) has a wide range of physiological functions such as binding, transport and distribution of biologically active compounds (Skrt et al., 2012). Thus, several studies (Skrt et al., 2012; Soares et al., 2007) explored the BSA binding mechanisms to polyphenols including tannins. Unlike fish-derived collagen, BSA denatures at around 60°C (Borzova et al., 2016) giving it an advantage over the isinglass in applications involving temperature. Therefore, BSA was used as another alternative method to remove phenolics from the extract.  **Research Question / Objectives / Hypothesis**  **Research Questions:**   1. Which of the tested seaweed polysaccharides extraction methods (year 1) is the most effective? 2. What is the most efficient, food- friendly way to separate polysaccharides from the co-extracted phenolic compounds? 3. Can *Ascophyllum nodosum* and *Saccharina latissima* serve as a source for the food-fibre production? 4. Can the technique of separation polysaccharides from the co-extracted phenolic compounds be successfully implemented into industrial-scale seaweed food-fibre production?   The focus during the second year of the PhD is to identify the most optimal technique for the polysaccharides extraction through evaluating the results from the experiment conducted over the first year. Another goal was to perform series of extractions using a chosen technique and develop a method to separate seaweed-derived polysaccharides and polyphenols. Obtained knowledge then is applied into seaweed-based food-fiber production during my industry placement at CyberColloids Ltd. (May-October 2022).  **Objectives:**   1. To evaluate the content of the extracts from the first year of PhD and identify the most effective extraction technique; 2. to develop an efficient and food-friendly way to separate polysaccharides from the co-extracted phenolic compounds; 3. to develop a protocol for the food-fibre production from *Ascophyllum nodosum* and *Saccharina latissimi*; 4. to implement the developed (Objective 2) polysaccharide-phenolics separation technique into food-fibre production.   **1 st Hypothesis:** The optimization of solvents, their ratio to the seaweed material, temperature variation, and time of exposure will increase the amount of extracted components from seaweed.  **2 nd Hypothesis:** Food-friendly chemicals at certain concentration and other specific conditions added within the polysaccharides extraction process can bind and precipitate phenolic compounds.  **2. Methodology**   * 1. **Material collection and preparation**  **2.1.1 Material collection**    1. ***b)***   ***Figure 1. Ascophyllum nodosum* collection site on Google maps.**  *Ascophyllum nodosum* was collected at a low tide on 7th of October 2020, 28th of April 2021 at Spiddal Harbour (53.2416360, -9.3101280), County Galway, Ireland (Figure 1 and Figure 3). *Saccharina latissima* was collected at a low tide on 8th of October 2021, at Finavarra Harbour (53.156000, -9.120000), County Clare, Ireland (Figure 2 and Figure 4).   1. ***b)***   ***Figure 2. Saccharina latissima* collection site on Google maps.**  **b)**      ***Figure 3.* Seaweed collection at Spiddal Harbour on 28th of April 2021 a) PhD student Sofiia Tretiak with harvested *Ascophyllum*; b) close look at the harvested *Ascophyllum*.**      ***Figure 4.* Seaweed collection at Finevarra Harbour on 8th of October 2021: close look at the harvested *Saccharina latissima*.** **2.1.2 Material preparation:** *Ascophyllum nodosum* and *Saccharina latissima* were washed with tap water, long piecies of thallus where chopped into smaller pieces (Figure 5a). After that the material was frozen at -18°C, then freeze-dried using Labconco FreeZone 6 freeze drier (Labconco Corp., Kansas City, MO, USA) (to ensure that all batches remained identical even if processed on different dates) and milled using the coffee grinder (Figure 5b). Milled material was kept at 4°C until further use.  **b)**  **a)**  ***Figure 5.* Seaweed material preparation a) *chopped Saccharina latissima;* b) milled *Ascophyllum nodosum.***  . Experiment 1**Experiment 1 Setup (a quick reminder from what has been done during the year 1)** Experiment 1 was set up to compare and select the most effective method for polysaccharide extraction. In this experiment 8 different extraction treatments were tested with 3 different solvents (Figure 6). The extraction was performed with 80% ethanol (Foley et al., 2011; Palanisamy et al., 2017; Rioux et al., 2007), distilled water (Allahgholi et al., 2020; Yuan & Macquarrie, 2015a) and 0.1 M hydrochloric acid (HCl 0.1 M) (Garcia-Vaquero et al., 2019).  The second phase of extraction included extraction with either distilled water (Foley et al., 2011; Rioux et al., 2007) or 0.1 M hydrochloric acid as solvents (Fletcher et al., 2017a; Yuan & Macquarrie, 2015a) at 70°C (Figure 7).  A picture containing timeline  Description automatically generated  ***Figure 6.* Experiment 1 Setup: Extraction Phase 1 scheme.**  ***A picture containing diagram  Description automatically generated***  ***Figure 7.* Experiment 1 Setup: Extraction Phase 2 scheme.** **Experiment 1: biochemical assays and evaluation of the results.** Total phenolic content was evaluated using the Folin Ciocalteu method adapted by (Zhang et al., 2006) with some modifications. The calibration curve was conducted with a range (0–100 *μ*g mL-1 (*R* ≥ 0*.*998) of phloroglucinol solution dilutions. Total sugar content was performed using the Dubois essay (Dubois et al., 1956) modified by (Singleton et al., 1999). The calibration curve was conducted with a range (0–100 *μ*g mL-1 (*R* ≥ 0*.*997) of glucose solution dilutions. Protein levels were quantified using the Bradford method of protein quantification (Bradford, 1976) with some modifications. The calibration curve was performed with a range (0–500 *μ*g mL-1 (*R* ≥ 0*.*998) of BSA solution dilutions.  The content of polysaccharides, phenolics and proteins is demonstrated in the Figure 8.  ***Ethanol 80%***  ***H2O + autoclave***  ***HCl 0.1 M + autoclave***  ***H2O + microwave***  ***HCl 0.1 M + microwave***  ***Control (no pre-treatment)***  ***Phase 2 extraction solvent***  ***Figure 8.* The percentage of polysaccharides, phenolics, and proteins in the extracts from Experiment 1.** The polysaccharides extracted in the experiment 1 were mainly fucoidans and laminarans, however, the further analyses of the sugar residues will be conducted. For this antibodies will be used in order to distinguish between fucoidans and laminarans content in extract. The extracted polysaccharides constituted from 9.89 to almost 30% of extract, where the lowest percent (9.89 + 0.59 %) of polysaccharides was in the samples from the Treatment 4 HCl 0.1 M – hydrothermal autoclave assisted pre-treatment during the phase 1 and with hydrochloric acid extraction during phase 2 of extraction. The highest percent (28.98 + 1.849) of polysaccharides was in extract Treatment 7 HCl 0.1 M – microwave assisted extraction during the phase 1 and with hydrochloric acid extraction during phase 2 of extraction.The ratio of polysaccharides to polyphenols were the lowest in both Treatments 6 (0.605, 0.348) using water solvent and microwave for the phase 1 and with both water and 0.1 M hydrochloric acid during phase 1 respectively. This means that the amount of co-extracted polyphenolic compounds were the highest in the samples of Treatment 6 (Treatment 6 – H2O, Treatment 6 HCl on the Figure 8).The highest ratio value was in the samples Treatment 1 HCl 0.1 M (3.374) and samples Treatment 3 HCl 0.1 M (3.959) that corresponded to ethanol 80% pre-treatment with 1 incubation and 3 incubation cycles respectively. However, the best methodology for the polysaccharides extraction is not a very straight forward decision to make. The procedure and solvents used is highly dependent on the scale and availability for the industrial – scale extraction.Experiment 2 planning**2.3.1 Experiment 2 Setup** Experiment 2 was set up to develop, compare and select the most effective method for polysaccharide-phenolics separation. In this experiment 9 different extraction treatments were tested with 3 potential phenolics binders (Table 1). All the extraction were performed with 80% ethanol (Foley et al., 2011; Palanisamy et al., 2017; Rioux et al., 2007) at the stage 1 of extraction and distilled water at the phase 2 (Treatment 1 H2O). The dosage for the PVPP treatment was 10 mg/mL of extracts as proposed by Toth & Pavia, 2001. The dosage for the BSA treatment for adjusted from the proposed dosage in the study of (S. Soares et al., 2007) and was equal to 500 mg/L and constituted 0.67% and 1.33 % of a total extract volume. The isinglass dosage was adjusted to what was suggested in the study of Yildirim, 2011 and added in the proportion where isinglass represented 1% of the total extract volume, concentration 2 g / L.  Prior to trying separation techniques in the real samples, the testing of the hypothesis was performed. Thus, various concentrations of phloroglucinol and alginic acid were chosen to mimic those in the real samples (0, 5 , 10, 30, 50, and 100 uL/mL water). At the highest concentration removal with PVPP was up to 95 % and with BSA above 90 %.   |  |  | | --- | --- | | **Polyvinylpolypyrrolidone (PVPP)** | **PVPP 1 dose (PVPP 1 D)** | | **PVPP 2 doses (PVPP 2 D)** | | **PVPP 1 dose at a time 3 times - total 3 doses (PVPP 1D+1D+1D)** | | **PVPP + filter** | | **Bovine Serum Albumin (BSA)** | **BSA 1 dose (BSA 1 D)** | | **BSA 2 doses (BSA 2 D)** | | **Isinglass** | **Isinglass 1 dose 12 h incubation, ph 7.0 (Isinglass 1 D, 12 h, ph 7.0)** | | **Isinglass 1 dose ph 4.4 (Isinglass 1 D, ph 4.4)** | | **Isinglass 2 doses ph 4.4 (Isinglass 2 D, ph 4.4)** | | **Control** | **Control 1 (phase 1 and phase 2 simultaneous precipitation)** | | **Control 2 (phase 1 phase 2 separate precipitation)** |   ***Table 1.* Different treatments for polysaccharides-phenolics separation**  **2.1.2 Experiment 2 results**  The added separation agent impacted a lot the total extract mass (Figure 9). Thus, the lowest weight was obtained in extracts from the BSA treatment where 2 doses of BSA were applied and the total extract yield was 35.0 + 2.8 mg. The highest yield was observed in both BSA 1 dose and Isinglass 1 dose treatments with weights 75.0 + 2 mg and 74.3 + 2.3 mg. However, this might be due to the protein residue in the extract sample as can be observed in the Figure 10.  ***Figure 9.* The total extract mass from different treatments.**  ***Figure 10.* The total extract mass from different treatments.**  Using an equation, the ranking system for the polysaccharide/phenolics treatments was developed. Thus, every treatment received a coefficient < 1 (Table 2). The equation considered the total weight of extract, the polysaccharides yield and the phenolics yields as well as eliminated the yield of proteins (as proteins remained in some of the extracts due to specific separation technique).   |  |  | | --- | --- | | **PVPP 2 D** | 1.00 | | **Isinglass 1 D, ph 4.4** | 0.85 | | **BSA 1 D** | 0.84 | | **Control 2 (phase 1 phase 2 simultaneous precipitation)** | 0.73 | | **PVPP 1 D** | 0.67 | | **Isinglass 2 D, ph 4.4** | 0.48 | | **Control 1 (phase 1 and phase 2 separate precipitation)** | 0.44 | | **PVPP + filter** | 0.38 | | **Isinglass 1 D 12 h incubation, ph 7.0** | 0.33 | | **PVPP 1D+1D+1D (total 3 D)** | 0.31 | | **BSA 2 D** | 0.18 |   ***Table 2.* The separation techniques placed from the most efficient to the least efficient using the coefficient.**  Therefore, the use of PVPP with 2 doses added to the extract was considered as the most efficient technique with almost 40% of polysaccharides and 2.76 % of phenolics content in the total extract (Figure 11). The BSA 2 doses the least efficient technique with almost 34.88 % of polysaccharides and 3.4 % of phenolics content in the total extract (Figure 11).  ***Figure 10.* The polysaccharides, phenolics, and protein content in the total extract expressed in % to the total extract weight.**   1. **Discussions**   The second experiment showed that the binding agent added into the extract is able to drastically change extraction yield. Moreover, the experiment held on alginic acid and phloroglucinol showed more efficient tares in phenolics removal suggesting that those polyphenols, identified after the separation experiment are bound to polysaccharides (Luo et al., 2020; Wu et al., 2022), and thus are more difficult to be eliminated from the solution. Following can be explained with the fact that free phenolics were removed during the first phase of extraction, where the ethanol was used as an organic solvent for phenolics removal, whereas the bound phenolics remained within cellulose and polysaccharides (Acosta-Estrada et al., 2014; Wu et al., 2022).  The fact that PVPP was the most efficient method for phenolics removal might be due to the fact that in wine industry PVPP is used specifically for tannins removal, and thus the nature of the process is very similar to the one in brown seaweed with phlorotannins removal. Moreover, PVPP is vegan-friendly and food-grade ingredient, thus its application for the seaweed-based food fibre production is very reasonable.  To summarise, a combination of soaking with organic solvent prior to extraction and adding a PVPP as phenolics binding agent are promising steps in phenolics content removal in food applications.   1. **References**   Abraham, R. E., Su, P., Puri, M., Raston, C. L., & Zhang, W. (2019). Optimisation of biorefinery production of alginate, fucoidan and laminarin from brown seaweed Durvillaea potatorum. *Algal Research*, *38*(November 2018), 101389. https://doi.org/10.1016/j.algal.2018.101389  Acosta, O., Vaillant, F., Pérez, A. M., & Dornier, M. (2014). Potential of ultrafiltration for separation and purification of ellagitannins in blackberry (Rubus adenotrichus Schltdl.) juice. *Separation and Purification Technology*, *125*, 120–125. https://doi.org/10.1016/j.seppur.2014.01.037  Acosta-Estrada, B. A., Gutiérrez-Uribe, J. A., & Serna-Saldívar, S. O. (2014). Bound phenolics in foods, a review. In *Food Chemistry* (Vol. 152, pp. 46–55). Elsevier Ltd. https://doi.org/10.1016/j.foodchem.2013.11.093  Ahmed, A. B. A., Adel, M., Karimi, P., & Peidayesh, M. (2014). Pharmaceutical, cosmeceutical, and traditional applications of marine carbohydrates. In *Advances in Food and Nutrition Research* (1st ed., Vol. 73). Elsevier Inc. https://doi.org/10.1016/B978-0-12-800268-1.00010-X  Akin, O., Temelli, F., & Köseoǧlu, S. (2012). Membrane Applications in Functional Foods and Nutraceuticals. *Critical Reviews in Food Science and Nutrition*, *52*(4), 347–371. https://doi.org/10.1080/10408398.2010.500240  Allahgholi, L., Sardari, R. R. R., Hakvåg, S., Ara, K. Z. G., Kristjansdottir, T., Aasen, I. M., Fridjonsson, O. H., Brautaset, T., Hreggvidsson, G. O., & Karlsson, E. N. (2020). Composition analysis and minimal treatments to solubilize polysaccharides from the brown seaweed Laminaria digitata for microbial growth of thermophiles. *Journal of Applied Phycology*, *32*(3), 1933–1947. https://doi.org/10.1007/s10811-020-02103-6  Becerra, M., Boutefnouchet, S., Córdoba, O., Vitorino, G. P., Brehu, L., Lamour, I., Laimay, F., Efstathiou, A., Smirlis, D., Michel, S., Kritsanida, M., Flores, M. L., & Grougnet, R. (2015). Antileishmanial activity of fucosterol recovered from Lessonia vadosa Searles (Lessoniaceae) by SFE, PSE and CPC. *Phytochemistry Letters*, *11*, 418–423. https://doi.org/10.1016/j.phytol.2014.12.019  Bhakti, T., & Avinash, M. (2019). Nutraceutical Potential of Seaweed Polysaccharides: Structure, Bioactivity, Safety, and Toxicity. *Comprehensive Reviews in Food Science and Food Safety*, *18*(3), 817–831. https://doi.org/10.1111/1541-4337.12441  Borzova, V. A., Markossian, K. A., Chebotareva, N. A., Kleymenov, S. Y., Poliansky, N. B., Muranov, K. O., Stein-Margolina, V. A., Shubin, V. v., Markov, D. I., & Kurganov, B. I. (2016). Kinetics of thermal denaturation andaggregation of bovine serum albumin. *PLoS ONE*, *11*(4). https://doi.org/10.1371/journal.pone.0153495  Bradford, M. M. (1976). A Rapid and Sensitive Method for the Quantitation of Microgram Quantities of Protein Utilizing the Principle of Protein-Dye Binding. In *ANALYTICAL BIOCHEMISTRY* (Vol. 72).  Calumpong, H. P., Maypa, A. P., & Magbanua, & M. (1999). Population and alginate yield and quality assessment of four Sargassum species in Negros Island, central Philippines. In *Hydrobiologia* (Vol. 398).  Choi, D. S. , Athukorala, Y. , Jeon, Y. J. , Senevirathne, M. , Cho, K. R. , & & Kim, S. H. (2007). Antioxidant activity of sulfated polysaccharides isolated from Sargassum fulvellum. *Preventive Nutrition and Food Science*, *12*(2), 65–73.  Cikoš, A. M., Jokić, S., Šubarić, D., & Jerković, I. (2018). Overview on the application of modern methods for the extraction of bioactive compounds from marine macroalgae. *Marine Drugs*, *16*(10). https://doi.org/10.3390/md16100348  Cunha, L., & Grenha, A. (2016). Sulfated seaweed polysaccharides as multifunctional materials in drug delivery applications. In *Marine Drugs* (Vol. 14, Issue 3). MDPI AG. https://doi.org/10.3390/md14030042  Dobrinčić, A., Balbino, S., Zorić, Z., Pedisić, S., Kovačević, D. B., Garofulić, I. E., & Dragović-Uzelac, V. (2020). Advanced technologies for the extraction of marine brown algal polysaccharides. *Marine Drugs*, *18*(3). https://doi.org/10.3390/md18030168  Doner, L. W., Bécard, G., & Irwin, P. L. (1993). *Food Cham* (Vol. 41). https://pubs.acs.org/sharingguidelines  Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A., & Smith, F. (1956). Colorimetric Method for Determination of Sugars and Related Substances. *Analytical Chemistry*, *28*(3), 350–356. https://doi.org/10.1021/ac60111a017  Fenoradosoa, T. A., Ali, G., Delattre, C., Laroche, C., Petit, E., Wadouachi, A., & Michaud, P. (2010). Extraction and characterization of an alginate from the brown seaweed Sargassum turbinarioides Grunow. *Journal of Applied Phycology*, *22*(2), 131–137. https://doi.org/10.1007/s10811-009-9432-y  Fletcher, H. R., Biller, P., Ross, A. B., & Adams, J. M. M. (2017a). The seasonal variation of fucoidan within three species of brown macroalgae. *Algal Research*, *22*, 79–86. https://doi.org/10.1016/j.algal.2016.10.015  Fletcher, H. R., Biller, P., Ross, A. B., & Adams, J. M. M. (2017b). The seasonal variation of fucoidan within three species of brown macroalgae. *Algal Research*, *22*, 79–86. https://doi.org/10.1016/j.algal.2016.10.015  Foley, S. A., Mulloy, B., & Tuohy, M. G. (2011). An unfractionated fucoidan from Ascophyllum nodosum: Extraction, characterization, and apoptotic effects in vitro. *Journal of Natural Products*, *74*(9), 1851–1861. https://doi.org/10.1021/np200124m  Garcia-Vaquero, M., O’Doherty, J. V., Tiwari, B. K., Sweeney, T., & Rajauria, G. (2019). Enhancing the extraction of polysaccharides and antioxidants from macroalgae using sequential hydrothermal-assisted extraction followed by ultrasound and thermal technologies. *Marine Drugs*, *17*(8). https://doi.org/10.3390/md17080457  Garcia-Vaquero, M., Rajauria, G., O’Doherty, J. V., & Sweeney, T. (2017). Polysaccharides from macroalgae: Recent advances, innovative technologies and challenges in extraction and purification. *Food Research International*, *99*, 1011–1020. https://doi.org/10.1016/j.foodres.2016.11.016  Garcia-Vaquero, M., Ummat, V., Tiwari, B., & Rajauria, G. (2020). Exploring ultrasound, microwave and ultrasound-microwave assisted extraction technologies to increase the extraction of bioactive compounds and antioxidants from brown macroalgae. *Marine Drugs*, *18*(3), 1–15. https://doi.org/10.3390/md18030172  Giacobbo, A., Moura, A., Norberta, M., & Pinho, D. (2017). Sequential pressure-driven membrane operations to recover and fractionate polyphenols and polysaccharides from second racking wine lees. *Separation and Purification Technology*, *173*, 49–54. https://doi.org/10.1016/j.seppur.2016.09.007  Gómez-Guzmán, M. , R.-N. A. , A. F. , & G. J. (2018). Potential role of seaweed polyphenols in cardiovascular-associated disorders. *Marine Drugs*, *16*(8).  Hentati, F., Delattre, C., Ursu, A. V., Desbrières, J., Le Cerf, D., Gardarin, C., Abdelkafi, S., Michaud, P., & Pierre, G. (2018). Structural characterization and antioxidant activity of water-soluble polysaccharides from the Tunisian brown seaweed Cystoseira compressa. *Carbohydrate Polymers*, *198*, 589–600. https://doi.org/10.1016/j.carbpol.2018.06.098  Hentati, F., Tounsi, L., Djomdi, D., Pierre, G., Delattre, C., Ursu, A. V., Fendri, I., Abdelkafi, S., & Michaud, P. (2020). Bioactive polysaccharides from seaweeds. *Molecules*, *25*(14), 1–29. https://doi.org/10.3390/molecules25143152  Hickman, D., Sims, T. J., Miles, C. A., Bailey, A. J., de Mari, M., & Koopmans, M. (2000). Isinglass/collagen: denaturation and functionality. In *Journal of Biotechnology* (Vol. 79). www.elsevier.com/locate/jbiotec  Kadam, S. U., O’Donnell, C. P., Rai, D. K., Hossain, M. B., Burgess, C. M., Walsh, D., & Tiwari, B. K. (2015). Laminarin from Irish brown seaweeds Ascophyllum nodosum and Laminaria hyperborea: Ultrasound assisted extraction, characterization and bioactivity. *Marine Drugs*, *13*(7), 4270–4280. https://doi.org/10.3390/md13074270  Kadam, S. U., Tiwari, B. K., & O’Donnell, C. P. (2013a). Application of novel extraction technologies for bioactives from marine algae. *Journal of Agricultural and Food Chemistry*, *61*(20), 4667–4675. https://doi.org/10.1021/jf400819p  Kadam, S. U., Tiwari, B. K., & O’Donnell, C. P. (2013b). Application of novel extraction technologies for bioactives from marine algae. In *Journal of Agricultural and Food Chemistry* (Vol. 61, Issue 20, pp. 4667–4675). https://doi.org/10.1021/jf400819p  Kadam, S. U., Tiwari, B. K., & O’Donnell, C. P. (2015). Extraction, structure and biofunctional activities of laminarin from brown algae. *International Journal of Food Science and Technology*, *50*(1), 24–31. https://doi.org/10.1111/ijfs.12692  Kravchenko, A. O., Barabanova, A. O. B., Glazunov, V. P., & Yakovleva, I. M. (2018). *Seasonal variations in a polysaccharide composition of Far Eastern red seaweed Ahnfeltiopsis flabelliformis ( Phyllophoraceae )*. 535–545. https://doi.org/10.1007/s10811-017-1262-8  Kuda, T., Yano, T., Matsuda, N., & Nishizawa, M. (2005). Inhibitory effects of laminaran and low molecular alginate against the putrefactive compounds produced by intestinal microflora in vitro and in rats. *Food Chemistry*, *91*(4), 745–749. https://doi.org/10.1016/j.foodchem.2004.06.047  Le Bourvellec, C., Bouchet, B., & Renard, C. M. G. C. (2005). Non-covalent interaction between procyanidins and apple cell wall material. Part III: Study on model polysaccharides. *Biochimica et Biophysica Acta - General Subjects*, *1725*(1), 10–18. https://doi.org/10.1016/j.bbagen.2005.06.004  Leather, R. v., Sisk, M., Dale, C. J., & Lyddiatt, A. (1994). ANALYSIS OF THE COLLAGEN AND TOTAL SOLUBLE NITROGEN CONTENT OF ISINGLASS FININGS BY POLARIMETRY. *Journal of the Institute of Brewing*, *100*(5), 331–334. https://doi.org/10.1002/j.2050-0416.1994.tb00831.x  Luo, M., Hu, K., Zeng, Q., Yang, X., Wang, Y., Dong, L., Huang, F., Zhang, R., & Su, D. (2020). Comparative analysis of the morphological property and chemical composition of soluble and insoluble dietary fiber with bound phenolic compounds from different algae. *Journal of Food Science*, *85*(11), 3843–3851. https://doi.org/10.1111/1750-3841.15502  Magalhães, P. J., Vieira, J. S., Gonçalves, L. M., Pacheco, J. G., Guido, L. F., & Barros, A. A. (2010). Isolation of phenolic compounds from hop extracts using polyvinylpolypyrrolidone: Characterization by high-performance liquid chromatography-diode array detection-electrospray tandem mass spectrometry. *Journal of Chromatography A*, *1217*(19), 3258–3268. https://doi.org/10.1016/j.chroma.2009.10.068  McMurrough, I., Madigan, D., & Smyth, M. R. (1995). Adsorption by Polyvinylpolypyrrolidone of Catechins and Proanthocyanidins from Beer. In *Food Chem* (Vol. 43). https://pubs.acs.org/sharingguidelines  Montes, L., Gisbert, M., Hinojosa, I., Sineiro, J., & Moreira, R. (2021). Impact of drying on the sodium alginate obtained after polyphenols ultrasound-assisted extraction from Ascophyllum nodosum seaweeds. *Carbohydrate Polymers*, *272*. https://doi.org/10.1016/j.carbpol.2021.118455  Otero, P., Quintana, S. E., Reglero, G., Fornari, T., & García-Risco, M. R. (2018). Pressurized Liquid Extraction (PLE) as an innovative green technology for the effective enrichment of galician algae extracts with high quality fatty acids and antimicrobial and antioxidant properties. *Marine Drugs*, *16*(5). https://doi.org/10.3390/md16050156  Palanisamy, S., Vinosha, M., Marudhupandi, T., Rajasekar, P., & Prabhu, N. M. (2017). Isolation of fucoidan from Sargassum polycystum brown algae: Structural characterization, in vitro antioxidant and anticancer activity. *International Journal of Biological Macromolecules*, *102*, 405–412. https://doi.org/10.1016/j.ijbiomac.2017.03.182  Pitkänen, L., Heinonen, M., & Mikkonen, K. S. (2018). Safety considerations of plant polysaccharides for food use: A case study on Phenolic-rich softwood galactoglucomannan extract. *Food and Function*, *9*(4), 1931–1943. https://doi.org/10.1039/c7fo01425b  Rajauria, G., Jaiswal, A. K., Abu-Ghannam, N., & Gupta, S. (2010). Effect of hydrothermal processing on colour, antioxidant and free radical scavenging capacities of edible Irish brown seaweeds. *International Journal of Food Science and Technology*, *45*(12), 2485–2493. https://doi.org/10.1111/j.1365-2621.2010.02449.x  Rhein-Knudsen, Nanna, M. T. A., & and Anne S. Meyer. (2015). Seaweed hydrocolloid production: an update on enzyme assisted extraction and modification technologies. *Marine Drugs*, *13*(6), 3340–3359.  Rioux, L. E., Turgeon, S. L., & Beaulieu, M. (2007). Characterization of polysaccharides extracted from brown seaweeds. *Carbohydrate Polymers*, *69*(3), 530–537. https://doi.org/10.1016/j.carbpol.2007.01.009  Siebert, K. J., & Lynn, P. Y. (1998). Comparison of polyphenol interactions with polyvinylpolypyrrolidone and haze-active protein. *Journal of the American Society of Brewing Chemists*, *56*(1), 24–31. https://doi.org/10.1094/asbcj-56-0024  Singleton, V. L., Orthofer, R., & Lamuela-Ravent6s, R. M. (1999). *Analysis of Total Phenols and Other Oxidation Substrates and Antioxidants by Means of Folin-Ciocalteu Reagent*.  Skrt, M., Benedik, E., Podlipnik, Č., & Ulrih, N. P. (2012). Interactions of different polyphenols with bovine serum albumin using fluorescence quenching and molecular docking. *Food Chemistry*, *135*(4), 2418–2424. https://doi.org/10.1016/j.foodchem.2012.06.114  Soares, S. , Mateus, N. , & & De Freitas, V. (2007). Interaction of different polyphenols with bovine serum albumin (BSA) and human salivary α-amylase (HSA) by fluorescence quenching. *Journal of Agricultural and Food Chemistry*, *55*(16), 6726–6735.  Soares, S., Mateus, N., & de Freitas, V. (2007). Interaction of different polyphenols with Bovine Serum Albumin (BSA) and Human Salivary α-Amylase (HSA) by fluorescence quenching. *Journal of Agricultural and Food Chemistry*, *55*(16), 6726–6735. https://doi.org/10.1021/jf070905x  Stengel, D. B., Connan, S., & Popper, Z. A. (2011a). Algal chemodiversity and bioactivity: Sources of natural variability and implications for commercial application. *Biotechnology Advances*, *29*(5), 483–501. https://doi.org/10.1016/j.biotechadv.2011.05.016  Stengel, D. B., Connan, S., & Popper, Z. A. (2011b). Algal chemodiversity and bioactivity: Sources of natural variability and implications for commercial application. In *Biotechnology Advances* (Vol. 29, Issue 5, pp. 483–501). https://doi.org/10.1016/j.biotechadv.2011.05.016  Stiger-Pouvreau, V., Bourgougnon, N., & Deslandes, E. (2016). Carbohydrates from Seaweeds. In *Seaweed in Health and Disease Prevention* (Issue 2010). Elsevier Inc. https://doi.org/10.1016/B978-0-12-802772-1.00008-7  Toth, G. B., & Pavia, H. (2001). Removal of dissolved brown algal phlorotannins using insoluble polyvinylpolypyrrolidone (PVPP). *Journal of Chemical Ecology*, *27*(9).  Wu, Y., Gao, H., Wang, Y., Peng, Z., Guo, Z., Ma, Y., Zhang, R., Zhang, M., Wu, Q., Xiao, J., & Zhong, Q. (2022). Effects of different extraction methods on contents, profiles, and antioxidant abilities of free and bound phenolics of Sargassum polycystum from the South China Sea. *Journal of Food Science*, *87*(3), 968–981. https://doi.org/10.1111/1750-3841.16051  Xu, S. Y., Huang, X., & Cheong, K. L. (2017). Recent advances in marine algae polysaccharides: Isolation, structure, and activities. *Marine Drugs*, *15*(12), 1–16. https://doi.org/10.3390/md15120388  Yildirim, H. K. (2011). Effects of fining agents on antioxidant capacity of red wines. *Journal of the Institute of Brewing*, *117*(1), 55–60. https://doi.org/10.1002/j.2050-0416.2011.tb00443.x  Yuan, Y., & Macquarrie, D. (2015a). Microwave assisted extraction of sulfated polysaccharides (fucoidan) from Ascophyllum nodosum and its antioxidant activity. *Carbohydrate Polymers*, *129*, 101–107. https://doi.org/10.1016/j.carbpol.2015.04.057  Yuan, Y., & Macquarrie, D. J. (2015b). Microwave assisted step-by-step process for the production of fucoidan, alginate sodium, sugars and biochar from Ascophyllum nodosum through a biorefinery concept. *Bioresource Technology*, *198*, 819–827. https://doi.org/10.1016/j.biortech.2015.09.090  Zhang, Q., Zhang, J., Shen, J., Silva, A., Dennis, D. A., & Barrow, C. J. (2006). A simple 96-well microplate method for estimation of total polyphenol content in seaweeds. *Journal of Applied Phycology*, *18*(3–5), 445–450. https://doi.org/10.1007/s10811-006-9048-4    *Enlarge this box as necessary.* |
|  |
|  |

|  |
| --- |
| **Indicate any communications of your work or relevant articles submitted for publication or published during this period:** |

|  |
| --- |
| **Completion Plan**  Students nearing completion must provide a completion plan  i.e.all full-time PhD/MD students in years 3, 4 and later (part-time students in years 4, 5, 6 and later) and all full-time Research Masters students in all years (part-time students in years 2 and later).  Plan must include tasks to be completed during the next year with timeframe.  A Gantt chart is appropriate.  Thesis writing should also be included along with publication plans.    **Plan for the Year 3**   1. To implement the developed polysaccharides-phenolics separation technique into industrial-scale food fiber production; 2. to explore food fiber production from the red seaweeds; 3. to develop a protocol for the food-fibre production from *Ascophyllum nodosum* and *Saccharina latissima*; 4. to extract alginate from the residue material from the experiment 1; 5. to evaluate M:G ratio in the alginate extracts using monoclonal antibodies 6. to scale up extraction of polysaccharides; 7. to test plant protection properties of extracts on strawberry plants; 8. IRC application (or other funding application).   *Enlarge this box as necessary.* |

|  |
| --- |
| When do you plan to submit your thesis?  By August 2024 (if the 4 years funding is granted), or February 2024 (if the 4 years funding is not granted) |

***Note:*** *Research students past their T****ime Limit*** *(i.e.* ***after*** *4 years for a full-time PhD, 6 year part-time PhD,* ***after*** *2 years for full-time Masters and 3 years part-time Research Masters students) should meet more frequently with their GRC e.g. quarterly.*

**For students on structured research programmes**

Complete the tables below indicating the taught modules you have taken this academic year.

|  |  |  |
| --- | --- | --- |
| **GS Modules to be assessed by supervisor**  (e.g. GS501) | | |
| **Code** | **Module Title** | **ECTS** |
|  |  |  |
|  |  |  |
|  |  |  |

|  |  |  |
| --- | --- | --- |
| **GS Modules (with module owners)**  (e.g. GS506) | | |
| **Code** | **Module Title** | **ECTS** |
| GS502 | Participation in the Journal Club Programme | 5 |
| GS5108 | Work-based placement 2 | 10 |
| GS508 | Formulating a Research Project Proposal | 5 |
| GS502 | Botany and Plant Science Seminar Series | 5 |
|  |  |  |

List of GS modules can be found at <http://www.nuigalway.ie/graduatestudies/module_table.html>

|  |  |  |
| --- | --- | --- |
| **Advanced Specialised Modules**  (generally discipline-specific, e.g. CH503) | | |
| **Code** | **Module Title** | **ECTS** |
| BI5108 | Green Lab Principles and Practice | 5 |
|  |  |  |
|  |  |  |

|  |
| --- |
| Have you successfully completed any module in another Irish university during this period?  If yes, provide details here and attach evidence of successful completion.  *It is important that you have registered for such modules at NUI Galway. College offices can advise on these procedures.* |

|  |
| --- |
| Have you completed the minimum number of taught modules required in your structured research programme?  If not, what modules do you plan to take next year?  *Module selection must be agreed with your supervisor.* |

Please attach to this report:

1. Your registration statement for this academic year indicating the modules for which you have registered (available from <http://www.nuigalway.ie/registration/>) and
2. For students in year 2 and later, copies of transcripts for earlier years of your research programme indicating the modules you have completed successfully (available from the Examinations Office).

|  |
| --- |
| **Student’s signature:** |

|  |
| --- |
| **Date: 13 June 2022** |

**University Guidelines for Research Degree Programmes**

<http://www.nuigalway.ie/graduatestudies/documents/university_guidelines_for_research_degree_programmes.pdf>